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Evidence for Persistence of Human Parvovirus B19 DNA in Bone Marrow

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A nested polymerase chain reaction assay (nPCR) was used to investigate the potential of human parvovirus B19 DNA to persist in blood or bone marrow samples obtained either from blood donors or cadaveric bone donors or from patients presenting with clinical signs of parvovirus B19 infection. The presence of parvovirus B19 specific antibody in blood was tested by enzyme immunoassay (EIA). B19 virus genome was not detected in any blood sample of 115 blood donors, of whom 92 (80%) had anti-B19 IgG antibody only as an indication of past infection. In contrast, B19 virus DNA was detected in the bone marrow of 4 out of 45 bone donors. Each one of the serum samples available for 3 of these 4 individuals contained anti-B19 IgG antibody. Among 84 patients with clinical manifestations of parvovirus B19 infection, 17 (20%) had B19 virus DNA in bone marrow. Eight of the latter patients had anti-B19 IgG antibody in their blood but neither anti-B19 IgM nor B19 virus DNA. These data document the ability of parvovirus B19 DNA to persist in the bone marrow of asymptomatic individuals and patients with parvovirus B19 infection suspected on clinical grounds. ***J. Med. Virol. 53:229–232, 1997.***

KEY WORDS: PCR; diagnosis; immunoassay; donors

INTRODUCTION

Human parvovirus B19, so far the only accepted member of the genus Erythrovirus in the subfamily Parvovirinae (Family Parvoviridae), was discovered by chance in 1975 [Cossart et al., 1975]. B19 replicates primarily in erythroid progenitor cells of the bone marrow which carry the erythrocyte blood group P antigen (or globoside) as the cellular receptor for B19 virus [Brown et al., 1993]. The first evidence of the pathogenic role played by parvovirus B19 in humans was obtained in 1980 in patients with a short febrile illness [Shneerson et al., 1980]. Subsequently, B19 was shown to be the agent of Erythema Infectiosum, a common febrile illness of childhood [Anderson et al., 1983]. Other common manifestations of parvovirus B19 infection are arthralgia and arthritis which are frequently observed in adults [White et al., 1985], non-immune fetal hydrops and fetal death secondary to transplacental infection [Brown et al., 1984], and transient aplastic crisis in patients with chronic hemolytic anemia [Pattison et al., 1981]. In the immunocompromised host parvovirus B19 infection may take a persistent course characterized by chronic anemia and pure red cell aplasia explained by the cellular tropism of B19 for erythroid precursors [Frickhofen et al., 1994; Erslev, Soltan, 1996].

There is well founded evidence that animal parvoviruses persist in a latent state [Siegl, 1988] and recent observations support persistence of parvovirus B19 DNA in immunocompetent individuals [Faden et al., 1992; Cassinotti et al., 1993a; Foto et al., 1993; Kerr et al., 1995; Sasaki et al., 1995; Söderlund et al., 1997]. In an attempt to gain more information on the persistence of parvovirus B19 DNA, a nested polymerase chain reaction (nPCR) assay was used to investigate its presence in blood and bone marrow cells obtained either from healthy donors or from patients presenting with clinical signs of parvovirus B19 infection.

SPECIMENS AND METHODS

Specimens

Blood donors. In order to study the prevalence of anti-B19 antibody and B19 DNA in the general population, blood samples were collected from 115 healthy adult blood donors showing no signs of acute parvovirus B19 infection.

Bone donors. Persistence of parvovirus B19 DNA was investigated in bone marrow samples obtained from 45 tendon or bone tissue donors deceased in the

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course of traffic accidents or cerebral hemorrhage. No other pathology was recorded. Corresponding blood samples collected at the same time as the bone marrow samples were available for 39 of the 45 donors. These blood samples were examined for the presence of anti-B19 antibody and viral DNA.

Patients with suspected parvovirus B19 infection. Bone marrow samples from 84 patients with parvovirus B19 infection suspected on clinical grounds were tested for the presence of B19 virus DNA. When available, blood specimens taken simultaneously were tested for the presence of anti-B19 antibody and B19 virus DNA.

B19 Virus DNA Extraction and PCR Amplification

B19 virus DNA was obtained from serum by heating and from bone marrow samples by treatment with proteinase K prior to nested PCR (nPCR) amplification [Cassinotti et al., 1993b]. In order to optimize speed and throughput, the original nPCR protocol designed for the TC-I thermocycler (Perkin Elmer, Foster city, CA) [Cassinotti et al., 1993b] was adapted to the GeneAmp PCR system 9600 (Perkin-Elmer) as follows: A fragment of the DNA sequence coding for the major capsid protein (VP2) of parvovirus B19 was amplified in a total reaction volume of 100 μl using the outer primers TJI (direct primer, nucleotides 3775 to 3792, 5′-TTC TTT TCA GCT TTT AGG-3′) and R4154 (reverse primer, nucleotides 4154 to 4171, 3′-TTT ATA CAG TGT CCT TAT-5′) numbered according to Shade and co-workers [Shade et al., 1986]. Following an initial incubation step at 94°C for 5 min the first amplification consisted of 35 cycles (denaturation at 94°C for 15 sec, annealing at 45°C for 60 sec, elongation at 72°C for 60 sec) terminated by a 10 min elongation step at 72°C. A 10 μl aliquot of the reaction product was used as substrate for a second amplification in a total volume of 100 μl with the inner nested primers 968 (direct primer, nucleotides 3818 to 3837, 5′-TAT AAG TTT CCT CCA GTG CC-3′) and TJII (reverse primer, nucleotides 3956 to 3975, 3′-GTA CTT CTG GTA CGT TAA GT-5′). The cycling parameters of the second amplification were the same as for the first amplification except for an annealing temperature of 60°C chosen to favor amplification of the inner fragment. Fifteen microliters of the reaction products were analyzed by agarose gel electrophoresis. The size of the diagnostic fragment amplified with the inner primers 968 and TJII was 157 base pairs. A 10^8-fold dilution of a viremic reference serum (N3787) containing approximately 10 to 100 parvovirus B19 genome copies was used as positive control. This PCR protocol has been used to test blood and bone marrow samples obtained from the bone donors. Most of the remaining samples were tested as described previously [Cassinotti et al., 1993b].

Serological Tests

The presence of anti-B19 IgM and IgG antibody was tested in serum samples by commercial enzyme immunoassays (EIA) based on the use of parvovirus B19 recombinant structure proteins (Parvovirus B19 IgM and IgG enzyme immunoassays, Biotrin Ltd, Dublin, Ireland).

TABLE I. Detection of Parvovirus B19 DNA in Bone Marrow Samples from Patients with Clinical Suspicion of B19 Virus Infection

B19-DNA	Number of bone marrow samples (%)	Number of patients (%)
Positive	18 (19%)	17 (20%)[a]
Negative	75 (81%)	67 (80%)
Total	93 (100%)	84 (100%)

[a]Clinical manifestations: Anemia in 7 patients (among them: 2 HIV positive, 1 solid organ transplant recipient), pancytopenia in 3 patients (among them: 1 bone marrow transplant recipient), arthritis in 2 patients, other manifestations or no information in 5 patients.

RESULTS

Incidence of Parvovirus B19-DNA and anti-B19 Antibody in Healthy Blood Donors

Possible persistence of parvovirus B19 DNA was examined by nPCR in the blood of 115 adult blood donors (54 women, 61 men, 21 to 66 years old) without clinical evidence of acute or recent parvovirus B19 infection. The presence of anti-B19 IgM and IgG antibody was assessed by commercial EIAs.

Ninety-two samples (80%) contained anti-B19 IgG antibody only as indicator of a past infection and anti-B19 IgM antibody was not detected in any sample. Parvovirus B19 genome was not detected by nested-PCR in any sample, thus suggesting that prolonged persistence of B19 DNA in the blood of otherwise healthy individuals is rare.

Detection of Parvovirus B19 DNA in Bone Marrow and Blood Samples from Patients with Clinical Suspicion of B19 Virus Infection and Correlation with Serological Markers in Blood

The presence of parvovirus B19 genome was investigated in a total of 93 bone marrow samples from 84 patients with B19 virus infection suspected on clinical grounds (Table I). Parvovirus B19-DNA was detected by nPCR in 18 samples from 17 patients (i.e. in 20%). The most frequent clinical manifestations observed in these 17 patients were related to the hematologic system. Anemia or pancytopenia were recorded in 7 and 3 patients, respectively. Of these 10 patients, two were infected with the human immunodeficiency virus (HIV) and 2 underwent solid organ or bone marrow transplantation. Rheumatologic manifestations such as arthropathy and chronic polyarthritis were observed as the sole manifestation in 2 patients.

A blood sample collected at the same time as the bone marrow sample was available for 12 of the 17 patients presenting with typical signs of parvovirus B19 infection and who had B19-DNA in their bone marrow. Of 3 patients with B19 DNA in their blood and bone marrow, one had anti-B19 IgM and IgG antibody, one had anti-B19 IgG antibody only and one had no

TABLE II. Detection of Parvovirus B19 Specific Antibody and/or DNA in Bone Marrow and Blood Samples Obtained from Bone Donors

Bone marrow DNA	Blood DNA	Number of patients	Serological status
Positive	Negative	3 (6.7%)	3 IgM− and IgG+
Positive	Positive	0 (0%)	
Positive	NA	1 (2.2%)	
Negative	Negative	36 (80%)	25 IgM− and IgG+
Negative	Positive	0 (0%)	11 IgM− and IgG−
Negative	NA	5 (11.1%)	
	Total	45 (100.0%)	

NA: blood sample not available.
IgM: anti-B19 IgM antibody.
IgG: anti-B19 IgG antibody.
−: negative.
+: positive.

anti-B19 antibodies. Among the 9 patients whose blood did not contain detectable levels of B19-DNA, one had anti-B19 IgM and IgG antibodies and 8 had anti-B19 IgG antibody only.

Detection of Parvovirus B19-DNA in Bone Marrow and Blood Samples Obtained from Bone Donors

In order to gain more information on the ability of parvovirus B19 DNA to persist in bone marrow following an acute infection, the presence of viral DNA was investigated in bone marrow samples obtained from a total of 45 healthy bone donors (Table II). Serum samples collected at the same time were tested for parvovirus B19 DNA and antibody. Whereas parvovirus B19-DNA was detected by PCR in bone marrow from 4 out of 45 donors (9%), it could not be detected in any of 39 serum samples available. Among the 39 serum samples available 28 (72%) contained anti-B19 IgG antibody as a sign of past infection and none contained anti-B19 IgM antibody. Furthermore, anti-B19 IgG antibody was detected in each serum sample available for 3 of the 4 individuals with B19-DNA in bone marrow. Thus, parvovirus B19 DNA may persist in the bone marrow of 11% (3/28) of the individuals with serologically documented past B19 virus infection, without leading to overt manifestations.

DISCUSSION

The ability to establish latent and persistent infection is a common characteristic of autonomous parvoviruses infecting animals [Siegl, 1988]. In humans, parvovirus persistence is well known for the dependovirus adeno-associated virus which integrates its genome in a site-specific manner in a locus on human chromosome 19 [Linden et al., 1996]. Infection of immunocompetent individuals with human parvovirus B19 is assumed to take a self-limited course followed by clearance of the virus from the host. It has been established, however, that, in some cases at least, persistent parvovirus B19 infections may occur, possibly causing pure red cell aplasia [Frickhofen et al., 1994]. Although such persistent infections have been described primarily in patients with congenital or acquired immunodeficiencies [Kurtzman et al., 1987; Gahr et al., 1991; Weiland et al., 1989; Musiani et al., 1995], PCR analyses have shown that they may also occur in immunocompetent individuals despite the presence of circulating anti-B19 antibody [Söderlund et al., 1997; Cassinotti et al., 1993a; Faden et al., 1992; Kerr et al., 1995; Sasaki et al., 1995; Foto et al., 1993].

In our study, persistence of parvovirus B19 DNA was not documented by nPCR in any blood sample of 115 blood donors, of whom 80% had anti-B19 IgG antibody only as a sign of past infection. In contrast, 11% (3/28) of healthy bone donors with serologically documented past B19 virus infection had B19 virus DNA in bone marrow, the main site of parvovirus B19 replication, but not in peripheral blood (Table II). These results suggest that, within the limit of sensitivity of the nPCR assay, prolonged presence of B19 virus DNA in the blood of otherwise healthy and asymptomatic persons with serological evidence of past parvovirus B19 infection is a rare event. However, at least a part of the viral genome seems to persist in bone marrow of a certain proportion of presumably healthy immunocompetent individuals who overcame acute B19 virus infection.

In addition, B19 virus DNA was detected in bone marrow from 20% (17/84) of patients with clinical manifestations of parvovirus B19 infection such as anemia or arthritis (Table I). A blood sample collected simultaneously with the bone marrow sample in the course of the symptomatic episode was available for 12 of the 17 patients. Four of these 12 blood samples contained B19 virus DNA and/or anti-B19 IgM antibody, a common pattern encountered in the course of acute infection. Anti-B19 IgG antibody but neither anti-B19 IgM antibody nor parvovirus B19 DNA were detected in the blood of 8 clinically ill patients who had viral DNA in bone marrow. Although an acute infection cannot be definitely ruled out, persistence of viral DNA seems more likely in these 8 patients since neither anti-B19 IgM antibody, which may be detected for up to 6 months [Cohen et al., 1983], nor B19 viral DNA were detected in the corresponding blood samples.

Reactivation of persistent infection might be a mechanism putatively accounting for the occurence of clinical symptoms in these patients. This hypothesis would have been substantiated by detection of viral DNA or antibody in blood or bone marrow samples collected before the onset of symptoms. Unfortunately, for those patients no such sample was available. Viral reactivation was suggested recently by Sasaki et al. [1995] during a course of persistent B19 virus infection in a normal person for whom B19 DNA was detected repeatedly in bone marrow several years after acute infection. Further evidence for reactivation of persistent parvovirus B19 infection has been discussed in the case of a 4-year old boy with acute lymphocytic leukemia [Coulombel et al., 1989].

In summary, the results presented in this report shed light on the potential of parvovirus B19 DNA to persist in the bone marrow of asymptomatic individuals as well as in patients presenting with typical symptoms of parvovirus B19 infection. Persistence of viral DNA seems to occur despite the presence of anti-B19 IgG antibody and without detectable levels of parvovirus B19 DNA in the circulating blood. At this stage it is unclear whether persistence of parvovirus B19 DNA is reflecting chronic infection or latent infection with intermittent episodes of viral reactivation. Further experiments are now needed to investigate the nature and clinical significance of parvovirus B19 DNA persistence in bone marrow.

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